Leica FISH Microscope Instructions

No Food or Drink Permitted in the Microscope Room
CRITICAL: The microscope can be seriously damaged by improper care of lenses. So review the following very carefully before turning on the microscope.

Lens care: Jarring a lens in any way can permanently destroy the alignment of the elements within it. Handle lenses very gingerly. Before examining a sample, make sure there is no foreign material on the sample that could contaminate the lens surface. After use, clean lenses while in the nosepiece and only with lens paper. Fold the paper several times and rub it back and forth over the lens surface. Use several pieces of lens paper until there is no longer evidence of oil on the paper. Avoid pressing the lens paper with your finger into the transparent part of the lens. If the lens becomes contaminated with any substance, contact the facilities manager for instructions on cleaning. Don't even think of applying any solvents to the lens surface.

What are the advantages of this imaging system?

This imaging system consists of fluorescence wide-field upright microscope, sensitive monochromatic 12-bit camera and a Metamorph software. With this system one may image in multiple channels, do time lapse imaging, create overlay images with adjusted brightness and contrast, modify the images and save them as 8 bit, 12 bit and 24bitRGB TIFF files suitable for importing into other programs.

Filter cubes of this system
A=DAPI, SC2=FITC and GFP, SC3=Cy3 (also Rhodamine, DsRed), SC4=Cy3.5 (also Texas Red), SC5=Cy5, SC6=Cy5.5, SC7=YFP, SC8-CFP.

Turning on the system.

1.) Turn on the mercury lamp power supply. Due to high voltage this unit should be switched on first and switched off the last, otherwise the other parts of the system will be damaged.
2.) Turn on the microscope. The microscope switch is on the microscope stand, right side, 0/I.
3.) Turn on the SenSys Photometrics camera (The black box on the top of the mercury lamp power supply).
4.) Turn on the computer Leica Q500CW and its monitor, log on and start the program Metamorph Leica.

Shutting down the system (If the system will be used within two hours, then only wipe oil from the lenses. Leave everything else on. Sign the logbook. Turn off the room lights)

Move your files to the Terabyte server 41_SSA_G1 /LRBGEIMAGE/YourFolder. This server will be automatically mounted on all the computers of the facility. If you will not see this server mapped on your personal computers, map it yourself. Find red N in the lower right corner of the computer screen, right-click on it. Select Novell Map Network Drive and for the "network path to resource" field, use the following syntax \\lrbgeimage.nci.nih.gov\ssa_41_g1\lrbgeimage.

1.) Exit the program. Switch off the computer
2.) Switch off the monitor
3.) Switch off the camera
4.) Put the objective in the highest position. Wipe off the oil from the objective. Switch off the microscope.
5.) Cover the microscope with plastic cover.
6.) Sign the logbook and note any problems.
7.) Turn off the room lights, and check once more if any microscope-associated lights remain on.

Viewing the slides.

The plunger on the left side of the eyepieces should be pushed in all the way. Vis – all the light goes to the eyepieces. Photo – all the light goes to the camera. 50/50 – exactly 50:50, do not use.

The objectives are changed manually. The magnification is displayed in the lower left corner of the display.

If you are using immersion oil, apply a small drop and put the slide on the stage. Do not mix immersion oils. If your slide has oil from another bottle, clean it first. If there is any other contaminant on your slide, clean it, or do not use that slide. Contaminating the lens surface is a very serious problem. Place your sample on the stage and then carefully raise the stage using the upper black button from the pair of square black buttons on the right side of the microscope next to the fine focus knob. Those buttons control the coarse focus, they move the stage in steps, not continuously, as in case of the regular mechanical focus common on other microscopes. If using an oil lens, raise the stage until the objective just flattens out the oil as the objective contacts your slide. Then observe the specimen through the oculars. DO NOT USE THE SIMILAR BLACK BUTTONS ON THE LEFT SIDE OF THE MICROSCOPE. They serve a different purpose.

You can use the fine focus knob now to gradually bring your specimen into focus. Check your slide periodically to make sure you are not over-focusing and pushing up the objective with the slide. This can seriously damage the lens. The fine focus has three steps from the coarsest to the finest. The button labeled Step, which is on the right side above the fine focus knob, allows one to change the step size for the fine focus. DO NOT USE THE SIMILAR BLACK BUTTON ON THE LEFT SIDE OF THE MICROSCOPE. It serves a different purpose. One can see which step one is using (S1 to S3) in the middle position of the upper lane of the display. S1 is 0.1 mkm, S3 is 1.5 mkm. Because fine focus is also changed in steps, not continuously, use S3 to bring your specimen in focus at the beginning of observation. Later use S1 to fine-tune your focus while imaging.

Transmitted light:

The switch with two arrowheads is located to the left of the main microscope switch. To view the specimen in transmitted light push it downwards. Dial on lower left in front of the fine focusing knob controls intensity of the transmitted light. The voltage is displayed in the upper left corner of the display while one is turning this dial. For fluorescence, turn off the transmitted light by turning the black dial away from you on the front left bottom of the microscope. Turn away the dial from you until the display panel on the front of the microscope reads 0*V. On the left side of the microscope, right at the bottom one may find both the field diaphragm and aperture diaphragm controls labeled “F” and “A”.

Fluorescence:

The switch with two arrowheads is located to the left of the main microscope switch. To view the specimen in fluorescent light push it upwards.

The filter cubes are changed automatically. To change the filter cubes use either the pair of black buttons behind the fine focus knob on the LEFT side of the microscope, or the two buttons labeled Fluor on the right upper side of the microscope below the camera and eyepieces. One can read which filter is in place (SC1 to 8) in the lower lane of the display.

The shutter for fluorescent light may be opened by pushing the black button labeled ‘shutter closed’. This button is behind the two buttons labeled ’Fluor’. If the shutter is closed, the red light next to the shutter button is on.

This microscope has both field and aperture diaphragms in the fluorescent light path. The levers for them are behind the filter and shutter buttons. If you are uncertain about them, please, ask Tatiana. Reduction of the field stop reduces out-of-focus light. Reduction of the aperture stop reduces the bleaching.

Metamorph Leica Instructions

The advantage of this program is that it collects 12 bit data saving them in a 16 bit format. You may scale these data for better viewing without loss of the original 12 bit data. This is important if you would like to quantify the fluorescence or compare samples with differing brightness. Below you will find instructions for the image acquisition and saving your data.

Starting the program

Double-click on icon Metamorph Leica. If you see no icon on the desktop, click on the icon MetaImaging Series. A window with different icons. Right-click on Metamorph Leica icon in the window and copy and paste it to the desktop.

Adjusting the settings and obtaining single images

First, you want to adjust the settings for your images. Click on the bar "Acquire Image Dialog" on a taskbar. The Acquire Image Dialog will open.

Now click on "Set to…" bar to set the acquisition to the appropriate channel setting. (For instance, "Set to FITC" if you want to adjust FITC). DO NOT use the "setting" choices within the Acquire Image window. Always select the appropriate setting with the bar on a taskbar.

After that you may modify your settings. When you made a change, hit "acquire" button in the window and check whether you like the quality of the image. If you do, then hit "Save" button in the window. If you changed something incorrectly accidentally and you do not know how to correct it, just hit the bar "Reset to Leica defaults".

You may modify the exposure. If your signal is very weak you may consider changing binning or gain - see the staff if you want suggestions on how to use these features.

You may also uncheck the "autoscale" and set the scaling to 0-4095 range. Autoscale leads to bright display of the images even if they are dim. So if you want to compare images, you have to scale them manually using the same scaling range. Consult the staff on best usage of this feature. You may later make the display of your images brighter or dimmer to view the details better, using the Display/Adjust Digital Contrast or Display/Scale Image dialogs. See below in "Saving Images" and "Saving Images for Adobe Photoshop".

If you are acquiring images in a single channel, you may continue using the button "Acquire". If you want to use multiple channels, see the following.

Obtaining sets of images in multiple colors

After you have adjusted and saved the settings, you may set the multichannel sequence for imaging.

Click on "Initial Acquire" bar. The window will open where you will check the channels you want to use. After that the images will be acquired in channels selected with your adjusted settings. This bar should be used each time you want to change the selection of the channels. I.e. if you have imaged slides stained with FITC and TRITC and now you wish to collect images in CFP and YFP channel, you have to select this new sequence of channels by using the "Initial acquire" bar.

To continue to use the same sequence of channels, hit "Acquire Again" bar. It will continue acquiring images with the chosen sequence of channels and acquisition settings. If you want to change the acquisition settings, chose the appropriate channels with "Set to..." bars, change them and "Save". After that hit "Acquire Again" and the images will be collected with the new settings.

To learn about the imaging conditions used to collect a certain image, hit the bar "Image Info Dialog".

Creating overlays

After you collected images, you may create overlay by hitting Color Combine bar. The window that will open is preset to create 24 bit RGB images that can be opened in Adobe Photoshop. Other choices (8 bit, 36 bit) may be used only in specific cases - see staff for explanations. So you need only to assign appropriate images to appropriate color channels (Red, Green or Blue). If you will click on buttons next to

channel choices you will see a list of files and you may choose the appropriate one. Then click on "Color Combine" button, and the overlay will appear in a new Color Combine window. Click "Save", and save this overlay with a default format TIFF. This will ensure that the overlay will be opened in Photoshop.

Viewing images

If you want to view the details better, you may change the brightness and the contrast of the image using Display/ Adjust digital contrast command. For 16 bit images any changes in this window will affect only the way the 16 bit image is displayed. They will not change the information of the image. For 8 bit images one may use "Fix the contrast" button and then save this image with the fixed new contrast and brightness settings.

You may also use Display/Scale Image dialog to change the scaling of the 16 bit image (another way of making images appear brighter) and to learn about the Min and Max values of the image. Scaling does not affect the image contents, it may be used only for display purposes. However, this dialog may be used to scale 16 bit images to 8 bit - see below "Saving Images for Adobe Photoshop". During this scaling the info of the new 8 bit image will be modified according to the chosen scale range.

One may assign color to the image by using the button within the image window.

Saving images

It is very important that you save your files as 16bit images. The camera collects 12 bit images that are recorded as 16 bit files. These files contain the complete unscaled info. Regardless of how your 16bit images are scaled on the screen, this scaling is only for display purposes, and the image information stays intact. 16 bit files will allow you to compare images collected with the same exposure and to quantify them. To save your images as 16 bit files, use "Save" button and the default TIFF format.

If you want to view the details better, you may change the brightness and the contrast of the image using Display/ Adjust digital contrast command. Any changes in this window will affect only the way the 16 bit image is displayed. They will not change the information of the image.

Saving images for Adobe Photoshop

Adobe Photoshop and Corel Draw use 8 bit images. Use Display/Scale Image command to convert your images to 8 bit images with or w/o scaling. In this dialog you may choose the correct scaling, that will be recorded when your image will be converted to 8 bit. During scaling you may lose some information if the scaling was done incorrectly. Please, ask the staff about the best usage of the scaling feature. Click on "Copy" button within this dialog and the scaled 8 bit image will be displayed. You may save this image ("Save") using the default TIFF format setting.

You may assign color to this image by clicking on a button within the image window. You may save this image ("Save") using the default TIFF format setting. The color will be displayed in Photoshop.

You may change the brightness and contrast of this image using Display/ Adjust digital contrast command. For 8-bit images you may save the image with new brightness and contrast values, first clicking on button "Fix contrast", and then using "Save" or "Save as" commands.